Lymphocyte Subset Counts in COVID-19 Patients: A Meta-Analysis

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Abstract

A reduced peripheral blood absolute lymphocyte count with an elevated neutrophil count has been a consistent observation in hospitalized COVID-19 patients. In this brief meta-analysis, the reduction of lymphocyte subset counts in COVID-19 patients was investigated across 20 peer-reviewed studies meeting criteria for reporting lymphocyte subset counts and COVID-19 disease severity. CD4+ T cell, CD8+ T cell, B cell, NK cell and total lymphocyte cell counts all showed statistically significant reduction in patients with severe/critical COVID-19 disease compared to mild/moderate disease. T cell subsets showed the largest standardized magnitude of change. In some studies, multivariate analysis has shown that CD4 and/or CD8 T cells counts are independently predictive of patient outcomes.

Key terms: COVID-19; Immunophenotyping; Lymphocyte subset; T Cell subset, flow cytometry

Introduction

In December 2019, a series of patients with pneumonia of unknown etiology was reported in Wuhan, Hubei Province, China.[1,2] Within weeks, a novel coronavirus, now named Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), was identified as the cause of this disease. SARS-CoV-2, a member of the genus beta coronavirus, has spread globally, leading to a pandemic that has infected over 7 million people and caused over 400,000 deaths (as of June 7th, 2020) in over 180 countries/ regions. This new pandemic has disrupted the global economy and put an enormous strain on global health care systems. The disease has been designated as coronavirus disease 2019 (COVID-19) by the World Health Organization and often presents clinically as fever, fatigue, muscle pain, diarrhea, and pneumonia, and can cause death in severe cases. The severity of the disease has been shown to be related to age and the presence of co-morbidities, such as diabetes, obesity, and heart disease. Several reports coming out of China have revealed that patients with the most severe cases of COVID-19 have abnormalities in many laboratory parameters, such as elevated procalcitonin, lactate dehydrogenase, D-dimer, C-reactive protein, neutrophil counts and pro-inflammatory cytokines, such as interleukin-6.[3] Lymphopenia and thrombocytopenia are also associated with severe COVID-19 disease. There is a growing list of publications indicating that the assessment of lymphocyte subset counts, such as CD4 and CD8 T cells, B cells, and NK cells, may provide prognostic information for COVID-19 disease severity and convalescence when considered in conjunction with other clinical information.[4,5] To obtain a clearer picture of this emerging data, we performed a meta-analysis of studies that included measurement of lymphocyte subset counts and disease severity in patients hospitalized with COVID-19.

Methods

A PubMed search (https://pubmed.ncbi.nlm.nih.gov/) on May 23rd for "COVID-19 Lymphocyte" produced a list of 258 publications. These publications were examined to exclude those that did not report patient clinical characterization data and lymphocyte subset evaluations. There were 16

publications that evaluated lymphocyte subset counts in COVID-19 patients with well characterized degrees of disease severity. An additional 4 publications meeting these criteria were found using a Google search. CD4+ and/or CD8+ T cell counts from COVID-19 patients with different disease severity status were reported in all 20 publications, and 10 of them also included CD19+ B cell and CD16+CD56+ NK cell counts. These 20 peer-reviewed publications were selected for meta-analysis in this brief report.[6-25]

These publications compared the results of peripheral blood lymphocyte subset counts in patients with mild/moderate disease to those with severe/critical disease hospitalized in China with a diagnosis of COVID-19 pneumonia. For the majority of the publications, the lymphocyte subset count data was gleaned from patient data registry, and reagents and flow cytometry instruments used in the measurement were not disclosed. A few publications reported the use of commercial in vitro diagnostic (IVD) products and lab developed tests (Supplementary Material, Table S1). The disease status assignments varied across studies, including survival vs. non-survival, moderate vs. severe/critical, aggravation vs. non-aggravation, and critical vs. non-critical. For the meta-analysis, COVID-19 patients were categorized into two groups: Mild/Moderate and Severe/Critical. The criteria used in the selected publications were consolidated as follows: mild, survival, non-critical and patients with non-aggravation disease were all classified into the "Mild/Moderate" group; deceased, non-survival, critical and patients with disease aggravation were all classified into the "Severe/Critical" group.

Results

The 20 publications selected for meta-analysis included a total of 3017 subjects with CD4+ cell counts where 2311 were classified as "Mild/Moderate" (76.6%) and 706 were classified as "Severe/Critical" (23.4%). The sample sizes of subjects with CD4+ and CD8+ T cell counts per publication varied from 17 to 499, with 10 to 479 in the mild/moderate group and 5 to 105 in the severe/critical group. A smaller subset of patients in the dataset had absolute lymphocyte subset counts for B cells and NK cells

reported. For publications with median and IQR or range reported, the mean and standard deviation were extrapolated according to Wan et al.[26] There is an apparent outlier in reported lymphocyte counts in one publication with increased total lymphocyte counts but decreased CD4+ and CD8+ T cells counts in critical vs non-critical patients, and this outlier in lymphocyte count was removed from the data analysis.[16] The mean cell counts in "Mild/Moderate" and "Severe/Critical" COVID-19 groups reported in each publication are shown in **Figure 1**. The cell counts were consistently decreased in the Severe/Critical group, and the differences in the weighted mean value of cell counts between the two patient groups are statistically significant for all cell types. Of note, the fold changes between Mild/Moderate and Severe/Critical groups for mean CD4 and CD8 T-cell counts are larger than for mean B cell, NK cell and total lymphocyte cell counts.

Meta-analysis was performed to calculate the standardized mean difference (SMD) and the 95% confidence interval (95% CI) between the Mild/Moderate and Severe/Critical groups for total lymphocytes, CD4+ T cell, CD8+ T cell, CD19+ B cell and CD16+CD56+ NK cell counts. R version 3.6.1 (2019-07-05)[27] with metafor[28] package was used for the analysis. A random effects model was used to account for heterogeneity between publications. The SMD for each parameter is summarized in **Figure 2**. For all parameters, the lymphocyte subset absolute counts were found to be significantly lower, on average, in subjects in the Severe/Critical group vs. the absolute counts in the Mild/Moderate group.

These results suggest that lymphocyte subset absolute counts are linked to patient outcomes. While some of the meta-analysis publications specified that cell counts were measured at or near hospital admission and tracked outcomes such as ICU admission or death, there is a need to assess this more thoroughly. A literature search was performed on outcome-based studies that included multivariate analyses of laboratory measurements with at least one lymphocyte subset. Across these studies, CD4 or CD8 T cell counts were independently linked to key patient outcomes including mortality,[13] ICU admission,[29] viral clearance,[30] and recovery[31] **(see summary Table 1)**. In a prospective study of 179

patients with COVID-19 pneumonia, CD8 T cells ≤ 75 cell/μL were independently linked to and predictive of mortality[13]; other independent predictors included age > 65, cardiovascular or cerebrovascular disease, and cardiac troponin I > 0.05 ng/mL. In a retrospective study of 249 patients, CD4 T cell counts measured at the time of hospital admission were inversely correlated with subsequent ICU admission; [29] age was the only other independent predictor. In a study of 60 patients, decreased CD8 T cells one week post-treatment (as well as decreased B cells and increased CD4/CD8 ratios) were associated with poor treatment efficacy[30]; this study controlled for age, sex, oxygen inhalation, antiviral treatment, disease severity on admission, and use of corticosteroid and immune enhancers. In a study of 292 patients in which 66 recovered after treatment, the CD4 T cell count measured before treatment was the only parameter measured that predicted the length of time before viral RNA clearance.[31] These findings suggest that CD4 and CD8 T cell absolute counts may be valuable biomarkers in the prognosis of disease severity and recovery in COVID-19 patients.

Discussion

Since the outbreak of COVID-19 in December 2019, a remarkable amount of observational clinical data has been published within a relatively short period of time. Although our understanding of viral replication and clinical manifestation is still in the early stages, some consistent clinical characteristics of the disease are emerging. Systematic review and meta-analysis studies of several clinical parameters have revealed that the severity of COVID-19 disease correlates with low blood albumin,[32] hypertension,[33] thrombocytopenia[34] and increased blood levels of IL-6 or Procalcitonin.[35,36] A recent meta-analysis reported by Huang et al showed that lymphopenia correlates with several poor patient outcomes, including mortality, ARDS, ICU care and severe diseases.[37] COVID-19 disease severity has also been linked to the lymphocyte-to-neutrophil cell count ratio and lymphocyte-to-CRP (C-reactive protein) ratio.[38]

This meta-analysis shows that absolute counts of major lymphocyte subsets are significantly and substantially decreased in severe COVID-19 disease. The results remain consistent despite the differences in the definition of disease severity across the studies, the variations in blood specimen acquisition times, laboratory practices, and clinical care. Multivariate analyses reviewed here establish immune cell subset counts, particularly CD4+ and CD8+ T cell counts, as independent predictors of COVID-19 outcomes. A limitation of this analysis is that all the studies were performed in China, and COVID-19 is now a global pandemic.

The pathogenesis of COVID-19 is still under investigation, and the precise mechanism(s) of the observed reduction in lymphocyte subset counts in the peripheral blood of patients with severe disease remain to be fully elucidated. Similar immune cell depletion has been reported in SAR-CoV-1 and MERS patients.[39] It has been suggested that the reduction of immune cell counts in the peripheral blood during viral infection may be caused by the mobilization of immune cells to sites of infection, such as the lungs, and potentially by virus-induced destruction of T cells.[40] Future prospective studies designed to investigate the utility of lymphocyte subset measurements as prognostic biomarkers of disease severity, mortality, and response to treatment in patients infected with SARS-CoV-2 are strongly encouraged.

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Figure Legends and Table Titles

Figure 1. Mean cell counts for each individual lymphocyte subset in Mild/Moderate and Severe/Critical COVID-19 patients across articles. Each solid circle represents the mean cell count in one article and the size of the circle represents the relative sample size for the mean. Paired data points from the same article are connected. The weighted mean value of cell counts across all articles is also shown with 95% confidence interval (CI) based on the random variability across articles.

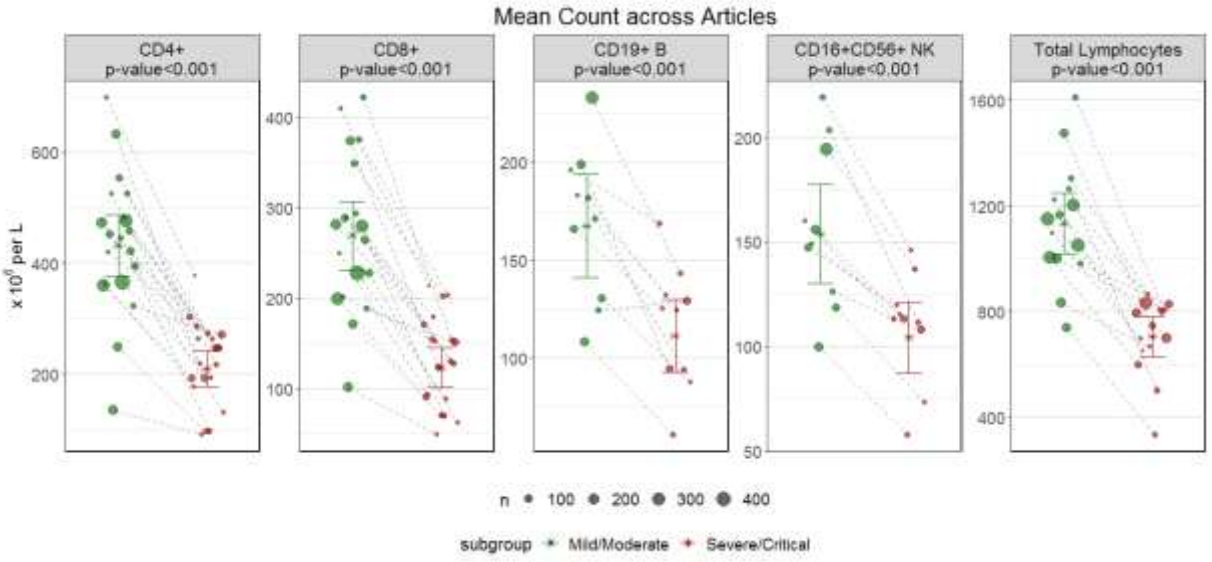
Figure 2. Standardized mean differences (SMD) between Mild/Moderate and Severe/Critical disease groups, with 95% confidence interval (95%CI), in COVID-19 patients. There is one panel for total lymphocytes, as well as panels for each subset. SMDs with CI results on the left of the 0 vertical line indicate a negative difference, i.e., the average mean count in the Severe/Critical group was significantly lower than in the Mild/Moderate group. Note that the width of the individual CIs is a function of sample size and reported variability within each publication.

Table 1: Lymphocyte subsets associated with COVID-19 outcomes in multi-variate analyses

Supplementary Material

Table S1: Study characteristic and demographics of patients in publications included in the meta-analysis

Figures:



	CD4+ T Cell		CD8+ T Cell		CD19+ B Cell		CD16+CD56+ NK Cell		Total Lymphocyte	
	Mild/ Moderate	Severe/ Critical	Mild/ Moderate	Severe/ Critical	Mild/ Moderate	Severe/ Critical	Mild/ Moderate	Severe/ Critical	Mild/ Moderate	Severe/ Critical
N	2311	706	2246	638	912	358	912	358	2124	1033
Average	432	210	269	123	167	111	154	104	1134	705
95% CI LB	377	178	232	101	141	93	130	87	1019	627
95% CI UB	487	241	307	145	194	130	177	121	1248	783
Fold Change	2.1		2.2		1.5		1.5		1.6	

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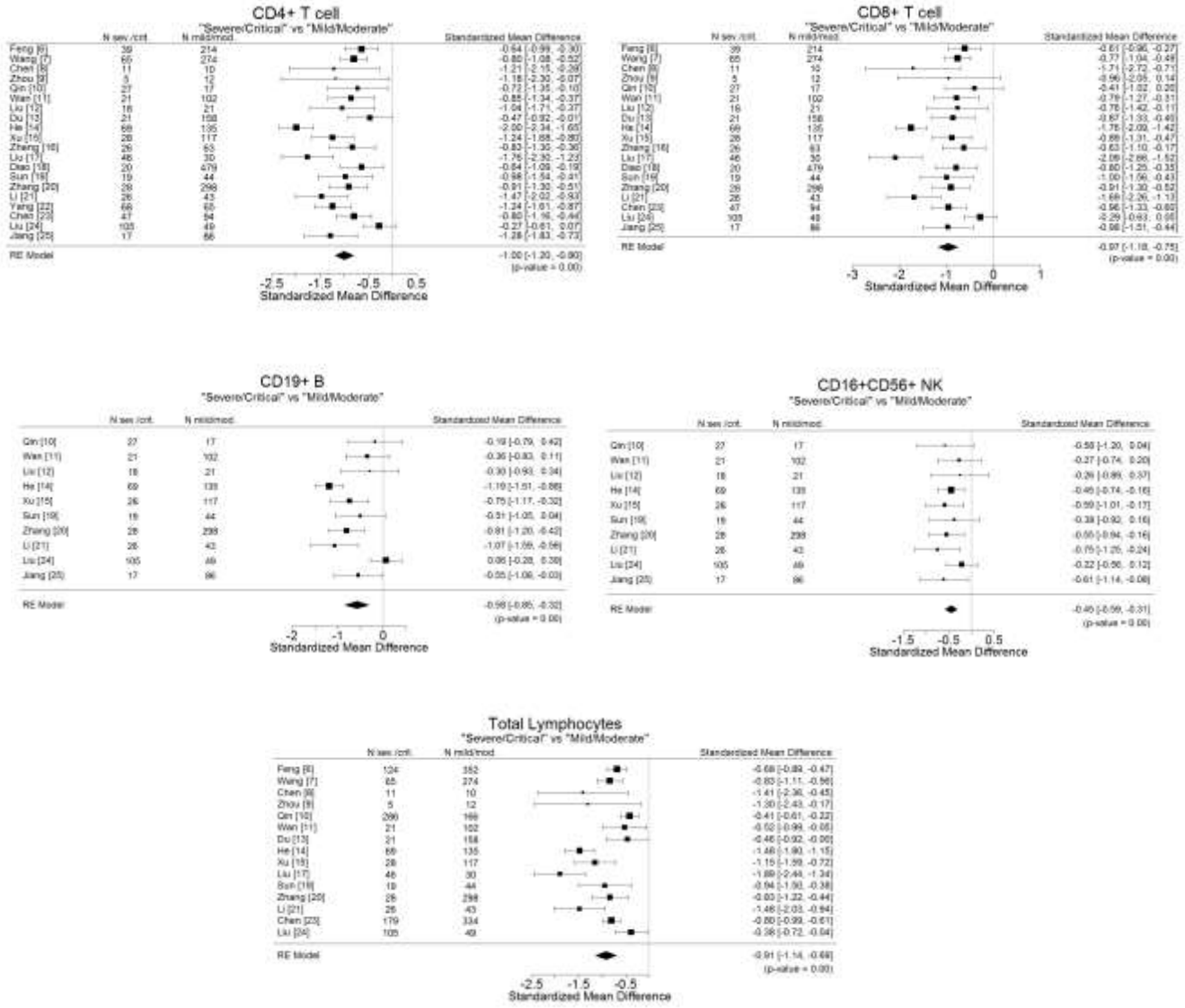

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Table 1: Lymphocyte subsets associated with COVID-19 outcomes in multi-variate analyses

Lymph subset	Outcome	Study Type	N	Statistical analysis (Odds Ratio, 95% CI, p value)	Ref
CD8 T	Mortality	Prospective	179 (21 died)	CD8 T cell < 75/μL (3.982, 1.132-14.006, <0.001)	Du [13]
CD4 T	ICU Admission	Retrospective	249 (22 admitted ICU)	CD4 T cell at hospital admission (0.55 per 100 cells/μL increase, 0.33-0.92, 0.02)	Chen [29]
CD8 T CD19 B	Treatment efficacy	Prospective	60 (37 responders)	Post-treatment decrease CD8 T (0.0056, 0.006-0.516, 0.011) CD19 B (0.033, 0.002-0.439, 0.010)	Wang [30]
CD4 T	Viral clearance (stool)	Retrospective	292, 66 recovered, 55 viral clearance (stool)	CD4 T cell (p=0.010)	Ling [31]

Supplementary Material

Table S1: Study characteristic and demographics of patients in publications included in the meta-analysis

Reference#	N	Gender, Male (N)	Gender, Female (N)	Age	Race/Ethnicity	Retrospective/ Prospective	#Hospitals	Disease Severity	Reagent/Flow Cytometer
Feng [6]	476	271	205	53 (IQR, 40-64)	Chinese	Retrospective	3	Mild, Severe, Critical	Not available
Wang [7]	339	166	173	71 ± 8	Chinese	Retrospective	1	Dead vs Survival	Not available
Chen [8]	21	17	4	56 (IQR 50–65)	Chinese	Retrospective	1	Severe vs Moderate	Lab developed test panels that include CD28, CD8, CD45, HLA-DR, CD3, CD45RA, CD45RO, CD127, CD25 and other BD reagents/FACSCanto II with FACSDiva
Zhou [9]	17	6	11	18 to 70	Chinese	Retrospective	1	Aggravation vs Non-aggravation	Not available
Qin [10]	452	286	166	58 (IQR 47-67)	Chinese	Retrospective	1	Severe vs Non severe	FACSCanto II (counts calculated from total lymph count)
Wan [11]	123	66	57	43.05±13.2 (Mild); 61.29±15.55 (Severe)	Chinese	Retrospective	1	Mild vs Severe	Panel 1 (CD3CD8/CD45/CD4) and Panel 2 (CD16+CD56/CD45/CD19)/Mindray BriCyte E6
Liu [12]	39	19	20	53 (IQR, 41–61)	Chinese	Retrospective	1	Mild vs Severe	Not available
Du [13]	179	97	83	57.6±13.7	Chinese	Prospective	1	Deceased vs Survival	Not available
He [14]	204	79	125	49 (IQR, 34-62)	Chinese	Retrospective	1	Severe vs Non-severe	Not available
Xu [15]	187	103	84	62 (IQR, 48.5-71)	Chinese	Retrospective	1	Discharged, Died, Stay in hospital	Not available
Zheng [16]	99	51	48	49.40±18.45	Chinese	Retrospective	1	Critical vs Non-critical	Not available
Liu [17]	76	49	27	45 (range 18-78)	Chinese	Retrospective	1	Mild vs Severe	Not available
Diao [18]	522	N/A	N/A	N/A	Chinese	Retrospective	2	ICU vs Non-ICU	BD Multitest™ IMK Kit/LSR Fortessa
Sun [19]	63	37	26	47 (range 3-85)	Chinese	N/A	N/A	Mild, Moderate, Severe, Critical	Not available
Zhang [20]	326	171	155	51 (range 15-88)	Chinese	N/A	1	Asymptomatic, Mild, Severe, Critical	BD Multitest™ 6-color TBNK reagent in Trucount tubes/BD FACSCanto II
Li [21]	69	40	29	Non-severe: 39 (IQR 29-53)	Chinese	N/A	N/A	Severe vs Non-severe	Not available
Yang [22]	133	72	61	Mild: 41.22±17.549 Severe: 59.97±14.126	Chinese	Retrospective	2	Mild vs Severe	Not available
Chen [23]	548	313	235	56.0±14.5	Chinese	Retrospective	N/A	Mild/Moderate, Severe, Critical	Not available
Liu [24]	154	84	70	64 ± 14	Chinese	N/A	1	Moderate, Severe, Critical	Multitest™ 6-color TBNK reagent/BD FACSCanto II
Jiang [25]	103	58	45	46 (range 17-88)	Chinese	N/A	1	Mild/Moderate, Severe	(FITC-CD4, PE-CD19, ECD-CD3, PC5-CD8, PC7-CD45)/Beckman Coulter Cytomics FC500